Carbon and Nitrogen Reserve Remobilization Following Defoliation: Nitrogen and Elevated CO₂ Effects

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ABSTRACT

Early regrowth following defoliation of forage species often depends on remobilization of nitrogen and non-structural carbohydrate (TNC) reserves stored in roots and crowns. The degree to which TNC and N remobilization contribute to regrowth can depend on internal concentrations and on external CO_2 and N supplies. We studied the effect of CO_2 and N supply on reserve remobilization during the first 20 d following defoliation of 9-wk-old alfalfa (*Medicago sativa* L.), western wheatgrass [*Pascopyrum smithii* (Rydb) A. Love], and blue grama [*Bouteloua gracilis* (H.B.K.) Lag ex Steud]. plants. Reserve remobilization was studied in controlled-environment chambers set at either ambient (350 µmol mol⁻¹) or elevated (700 µmol mol⁻¹) CO_2. Plants were fertilized twice weekly with Hoaglands solution containing either 0 mg L⁻¹ (low N) or 400 mg L⁻¹ N (high N). Elevated CO_2 increased the total amount and percent of available TNC that was remobilized in alfalfa, and the amount of remobilized TNC in western wheatgrass, but reduced TNC remobilization in blue grama. Nitrogen fertilization had little effect on TNC remobilization at ambient CO_2, but increased remobilization in alfalfa and reduced remobilization in the two grasses under elevated CO_2. Alfalfa remobilized a greater percentage of its root and crown N reserves than either grass species. Nitrogen remobilization was highest under high N and ambient CO_2 conditions for all species. Nitrogen deficiency and elevated CO_2 reduced N remobilization and the contribution of remobilized N to shoot regrowth.

NUTRIENT UPTAKE (Kim et al., 1993) and photosynthesis (Clement et al., 1978; Richards, 1993) can be greatly reduced following defoliation. As a result, early shoot regrowth often depends on remobilization of nitrogen and non-structural carbohydrate (TNC) reserves stored in roots and crowns (Davidson and Milthorpe, 1966; Chung and Trilca, 1980; Ourry et al., 1988; Baur-Hoch et al., 1990; Lefevre et al., 1991; Volenec et al., 1996). The degree to which TNC and N remobilization affect regrowth can depend on external CO_2 and N supplies and on plant reserve status. In several studies, nitrogen limitation reduced N uptake from the soil and the amount of stored N that was available for remobilization, but increased the proportion of N in regrowing leaves that had been remobilized from root and crown sources (Millard et al., 1990; Ourry et al., 1990; Thornton and Millard, 1993; Thornton et al., 1994). Even though reliance on remobilized N for regrowth increased under limited N supply, the rate of remobilization decreased (Millard et al., 1990; Thornton et al., 1994), suggesting

that N remobilization continued for a longer period of time when N supply was limited. However, when reserve N supplies were depleted by weekly defoliations, little or no N was remobilized from reserves, so that shoot growth relied solely on new uptake (Thornton and Millard, 1997).

The effects of elevated atmospheric CO_2 on plant growth and tissue composition have been extensively studied. However, few studies have examined the interaction between elevated CO_2 and defoliation. Elevated atmospheric CO_2 commonly increases TNC in plant tissues and reduces N concentration (Baur-Hoch et al., 1990; Korner and Miglietta, 1994; Wilsey, 1996). Carbohydrate remobilization after defoliation can also increase under elevated CO_2 (Baur-Hoch et al., 1990). In legumes, nitrogen fixation following defoliation was enhanced under elevated CO_2 (Ryle and Powell, 1992), which could have reduced the need for remobilized N to support regrowth. At present, however, the available data are much too limited to draw general conclusions about how defoliated plants respond to elevated CO_2. Studies are also needed which examine how the interaction between N supply and atmospheric CO_2 affects TNC and N remobilization following defoliation.

We have examined reserve remobilization and plant growth following defoliation in three functional plant types, a legume (alfalfa), a C_3 grass (western wheatgrass), and a C_4 grass (blue grama) which received limiting and non-limiting N supplies, and which were exposed to ambient and elevated atmospheric CO_2. These experimental conditions provided a wide range of root and crown TNC and N concentrations. Growth responses to N fertilization and elevated CO_2 will be described in detail elsewhere. To summarize, when the grasses received N fertilization whole plant growth rates were lowest immediately after defoliation then increased throughout the regrowth period. In the low N treatment, however, blue grama and western wheatgrass growth rates were highest immediately after cutting. These results were consistent with other studies in which growth was initially inhibited by defoliation under favorable conditions but not when nutrients are limited (Chapin and Slack, 1979; Millard et al., 1990, Richards, 1993). Alfalfa regrowth patterns were not affected by N treatment (Morgan, Skinner, and Hanson, 1998, unpublished data). Even though alfalfa seeds had not been inoculated with rhizobium, nodules were present in low N treatments, especially under elevated CO_2. Alfalfa, therefore, probably did not experience the same severity of N stress as did the two grasses. Elevated CO_2 generally had less of an effect on growth than did N fertilization. Regrowth was stimulated by elevated CO_2 in the

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Abbreviations: TNC, total nonstructural carbohydrates; SDW, Structural dry weight.

two C_3 species, alfalfa and western wheatgrass, but was inhibited in the C_4 species, blue grama. The purpose of this paper is to examine the effect of CO_2 and N supply on the initial content and remobilization of root and crown TNC and N reserves following defoliation of the same three plant species.

MATERIALS AND METHODS

Alfalfa, western wheatgrass, and blue grama seeds were planted in 15-cm diameter by 45-cm-deep polyvinylchloride pots containing a 50:50 sand:soil (Ascalon loam, fine loamy mixed mesic) mixture, and placed in a greenhouse under ambient CO_2 conditions. The greenhouse was maintained at approximately 22°C. Supplemental lighting was used when needed to maintain a 14-h photoperiod. After germination, plants were thinned to two seedlings per pot. Six weeks after planting, the seedlings were cut to a 5-cm stubble height and transferred to a walk-in growth chamber set at either ambient (350 μL L^{-1}) or elevated (700 μL L^{-1}) CO_2. The chamber had a 14-h photoperiod, 28/17°C day/night temperature, daytime relative humidity of ≈50%, and a photosynthetic photon flux density of about 700 μmol m^{-2} s^{-1}. The temperatures represented a compromise between the optima for C_3 and C_4 species, but in retrospect favored blue grama growth over that of western wheatgrass. Nutrient treatments were initiated 1 week after planting and consisted of 100 mL of half-strength Hoagland's solution containing either 0 or 400 mg L^{-1} N as NH$_4$NO$_3$, applied twice weekly. Pots were also watered as needed to avoid moisture stress.

Three weeks after placement in the growth chamber, plants were again cut to a 5-cm stubble height. The 5-cm cutting height removed all mature leaf blades from the two grasses, leaving only the sheaths and enclosed elongating blades. To ensure that new shoot growth could be distinguished from the stubble left after cutting, all aboveground growth from western wheatgrass rhizomes was cut at the soil surface, and all unfolded alfalfa leaves below 5 cm were removed leaving only stems and newly developing leaves. Sequential harvests were made at 0, 4, 7, 10, 14, and 20 d after cutting. Plants were separated into roots, crowns (including western wheatgrass rhizomes), and new shoot growth, immediately immersed in liquid nitrogen and freeze dried. Dried materials were then weighed to obtain estimates of root, crown, and shoot biomass.

Alfalfa regrowth included all leaves and stems above 5 cm plus all unfolded leaves below that height. Each grass tiller was harvested separately. Mature sheaths below 5 cm were included with crowns, while elongating leaves within those sheaths were placed with the new growth. All biomass from tillers which emerged after the clipping treatment was also included with the regrowth, regardless of height within the canopy. The distal 5 cm of each leaf blade that was elongating at the time of cutting was included with the crowns since those tissues were part of the stubble left after clipping.

Dry matter was partitioned into nitrogen containing compounds, structural dry matter and nonstructural carbohydrates. Buffer soluble and insoluble N were separated using 100 mM NaPO$_4$ buffer according to the procedure of Barber et al. (1996). Proteins were precipitated from the buffer soluble fraction with 720 μL mL^{-1} trichloroacetic acid. The resulting fractions included buffer soluble proteins, buffer insoluble N, and low molecular weight N compounds including amino acids, NO$_3$, and NH$_4$. Buffer insoluble N was quantified with a LECO C and N analyzer (LECO, St. Joseph, MI). Buffer soluble protein and low molecular weight N fractions were quantified by Kjeldahl analysis procedures. The low molecular weight N samples were pre-treated with salicylic acid and sodium thiosulfate pentahydrate to convert NO$_3$ to NH$_4$ prior to Kjeldahl digestion.

Nonstructural carbohydrates were determined by the method described by Hendrix (1993) which allows TNC to be partitioned into starch, sucrose, fructose, and glucose. Fructose and glucose fractions were quantified as a single pool, hereafter referred to as hexoses. Other glucose and fructose containing water soluble polysaccharides were quantified by boiling a subsample of the extracted sugars in 0.2 M acetic acid for 1.5 h. The acid was then neutralized with 1 M NaOH. This procedure breaks down the polysaccharides to glucose and fructose which can then be quantified by the procedure of Hendrix (1993), providing an estimate of total soluble sugars. The sucrose and hexose levels that had been determined separately were then subtracted from the total soluble sugars to provide an estimate of the other soluble sugars. In western wheatgrass, these other sugars would primarily be fructans which are the principle storage carbohydrates in C_3 grasses. Other sugars such as maltose probably predominated in alfalfa and blue grama, which do not store significant amounts of fructan. Non-structural carbohydrate and N pool sizes were determined by multiplying carbohydrate and N concentrations by total dry weight. The TNC, soluble protein, and low molecular weight N fractions were subtracted from total dry weight to obtain an estimate of structural dry weight (SDW).

Remobilization from root and crown tissues was determined for the soluble N (buffer soluble proteins and low molecular weight N) and TNC fractions. Remobilization was calculated as the difference between soluble N or TNC content at 0 or 4 d after defoliation (whichever was greater) and the minimum content, which was usually observed at 7 or 10 d after defoliation. Because we could not determine the actual fate of the TNC or soluble N that was lost from roots and crowns, remobilization was broadly defined to include all TNC or soluble N that was lost from those tissues.

Because only one growth chamber was available for the study the experiment was repeated four times, twice at 350 μmol mol^{-1} CO_2 and twice at 700 μmol mol^{-1} CO_2. Within each run, a factorial arrangement of three species, two N concentrations, six harvest dates, and three subsamples were randomly placed within the growth chamber for a total of 108 pots. The experiment was analyzed as a split-plot design with CO_2 treatments as the whole plots. Because greenhouse temperature and photoperiod were controlled, plants in each run were at essentially the same size and stage of development when placed in the growth chamber.

RESULTS AND DISCUSSION
Carbohydrate Remobilization

Reserve remobilization can be determined from net changes in TNC or N pools, or by following the movement of labeled compounds. A problem with following labeled tracers is that carbohydrates (Farrar, 1989) and proteins (Davies, 1982) are constantly turning over in plant tissues, regardless of defoliation treatment. This makes it difficult in labeling studies to distinguish between constitutive turnover and remobilization due to defoliation (De Visser et al., 1997). In this study, we were concerned with whether or not a net depletion of root and crown reserves had occurred in response to defoliation. Apparent reserve remobilization, therefore, was determined from the net loss of TNC and N from root and crown pools.

Elevated CO_2 increased TNC concentrations in the roots and crowns of all three species at the time of defoliation under both low and high N fertility treatments (Table 1). Nitrogen fertilization, however, had no significant effect on TNC concentrations, with the exception of western wheatgrass grown under elevated CO_2, where high N caused a reduction in root and crown TNC. In alfalfa and blue grama, the greatest effect of elevated CO_2 was on starch concentrations which increased between 70 and 290% when atmospheric CO_2 was increased. Western wheatgrass accumulated very little starch, and the increase in TNC concentration resulting from elevated CO_2 was evenly distributed among the carbohydrate fractions.

Root and crown TNC concentrations began to decrease immediately following defoliation in all species and treatments (Fig. 1). In some instances, however, the actual TNC content of roots and crowns remained constant for the first 4 d following defoliation before decreasing thereafter. In these cases, TNC concentrations decreased because roots and crowns continued to produce structural dry mater without an accompanying accumulation of TNC. This shift in partitioning of new assimilates between structural and nonstructural growth was then followed by remobilization of TNC reserves.

The effect of elevated CO_2 on TNC remobilization was species dependent. In alfalfa, elevated CO_2 increased both the total amount and the relative proportion of TNC that was remobilized (Table 2). In western wheatgrass, the amount of TNC that was remobilized was increased under elevated CO_2, while the relative proportion that was remobilized changed only slightly. In blue grama, however, both the total amount and the relative proportion of TNC that was remobilized decreased under elevated CO_2. Nitrogen fertilization had less effect than elevated CO_2 on TNC remobilization in all species. The greatest effects of N fertilization were on the amount of TNC that was remobilized under elevated CO_2 in alfalfa, and on the relative proportion remobilized in blue grama and western wheatgrass, again under elevated CO_2.

Blue grama regrowth following defoliation decreased under elevated compared with ambient CO_2 (Morgan, Skinner, and Hanson, 1998, unpublished data). As with the two C_3 species, TNC concentrations in the roots and crowns of blue grama under elevated CO_2 were significantly higher by 20 d after defoliation than they were at the time of cutting (Fig. 1). This increase was driven by a rapid accumulation of starch (data not shown). The accumulation of starch suggests that the growth inhibition experience by blue grama under elevated CO_2 was not primarily a result of reduced photosynthetic capacity, but instead, resulted from an inability to rapidly convert photosynthate to structural dry matter.

Several studies have shown an increase in shoot TNC, especially starch, concentrations under elevated CO_2 (Finn and Brun, 1982; Farrar and Williams, 1991; Korner and Miglietta, 1994; den Hertog et al., 1996), but the effect of atmospheric CO_2 on root carbohydrates is less clear. Increased, decreased and unchanged root TNC

Table 1. Concentration of individual root and crown carbohydrate fractions and of total nonstructural carbohydrates (TNS) at the time of defoliation. Concentrations are expressed on a structural dry weight (sdw) basis. Data within a row followed by the same letter are not significantly different at α = 0.05.

	Ambient CO_2		Elevated CO_2		
	Low N	High N	Low N	High N	$LSD_{0.05}$
	mg/g sdw				
Alfalfa					
Starch	194b	146b	330a	327a	100
Hexose	11b	10b	12b	16a	4
Sucrose	40b	36b	53a	51a	10
Other Sugars	27a	18b	27a	27a	7
TNC	271b	210b	421a	421a	106
Blue grama					
Starch	18bc	10c	45a	39ab	23
Hexose	16ab	13b	17a	16ab	4
Sucrose	20ab	14c	24a	16bc	5
Other Sugars	5a	1ab	0b	0b	5
TNC	58bc	38c	85a	70ab	24
Western wheatgrass					
Starch	5a	5a	14a	19a	NS
Hexose	17b	19b	30a	27a	5
Sucrose	21c	22c	38a	29b	6
Other Sugars	9b	11b	40a	14b	13
TNC	52c	57c	123a	90b	29

levels have all been reported, depending on species, temperature, and age of the plants (Baxter et al., 1995; den Hertog et al., 1996; Read and Morgan, 1996). Of particular interest is the study by Read and Morgan (1996) because they examined the same grass species as this study. Their plants were undefoliated, but otherwise grown under similar conditions as the current study. They found that blue grama root TNC concentrations were unaffected by elevated CO_2 while western wheatgrass concentrations increased, decreased, or were unchanged depending on temperature and plant age. In our study, atmospheric CO_2 concentration had a significant influence on how defoliation and subsequent regrowth affected root and crown TNC concentrations. When plants were grown at ambient CO_2, root and crown TNC concentrations had barely recovered to predefoliation levels by 20 d after cutting (Fig. 1). Under elevated CO_2, however, root and crown TNC concentrations at 20 d were, on average, 64% greater than they were at the time of defoliation. Thus, differences in TNC concentration between CO_2 treatments were much greater after defoliation than they were before.

A poor correlation often exists between regrowth and TNC concentration or remobilization (Volenec et al., 1996). We also failed to observe any significant relationship between regrowth and either initial TNC content, or the amount remobilized (data not shown). One reason might be that remobilized TNC has multiple potential fates. According to Avice et al. (1996), more than 90% of the TNC remobilized following defoliation was used for root and shoot respiration, with root respiration being the predominant sink. Root respiration can be divided into three main components which provide energy for growth, cellular maintenance, and nutrient uptake. Growth and maintenance respiration are roughly equal, and together account for 90% or more of total respiration (Bouma et al., 1996; Mata et al., 1996).

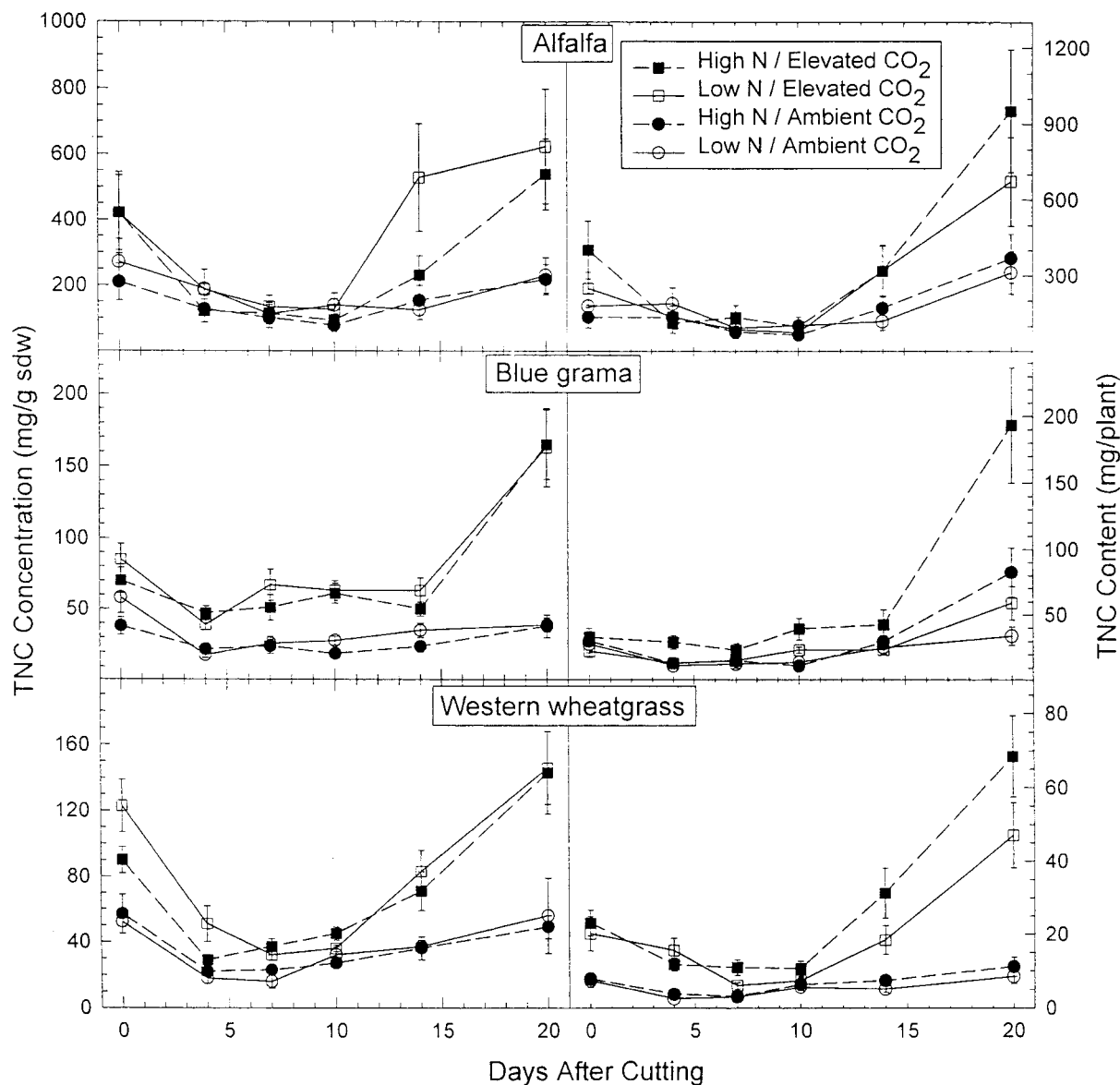This multitude of uses for remobilized TNC makes it

Fig. 1. Changes in the concentration and content of total nonstructural carbohydrate (TNC) reserves in the roots and crowns of alfalfa, blue grama, and western wheatgrass during the first 20 d following defoliation. Concentrations are expressed on a structural dry weight (sdw) basis. Error bars represent $\pm$1 SE.

unlikely that any one response, i.e., shoot dry matter accumulation, would be highly correlated with TNC remobilization. If the partitioning of remobilized TNC between growth and respiration was similar in our study to that found by Avice et al. (1996), then the maximum contribution of remobilization to shoot dry matter during the first 4 d following defoliation in any of our treatments would have been 17% in alfalfa grown at high N and elevated CO_2. In most treatments, remobilized TNC would have contributed <1% of the total shoot biomass during the same 4-d period.

Nitrogen Remobilization

Alfalfa had the greatest root and crown N concentrations at the time of defoliation, followed by western wheatgrass then blue grama (Table 3). Among the species tested, alfalfa N concentrations were least affected by the nitrogen and CO_2 treatments. Because alfalfa has

the ability to fix atmospheric N it may be less responsive to environmental conditions which affect soil N uptake. In alfalfa, the significant increase in total N concentration in the high N, ambient CO_2 treatment was primarily due to a 40% increase in low molecular weight N concentration compared with the other treatments.

Root and crown N concentrations for both grasses were higher in high compared with low N treatments, and were generally lower under elevated compared with ambient CO_2. The one exception was blue grama, where total N concentrations were similar under N fertilization regardless of CO_2 treatment. Again, the reduced growth under elevated CO_2 may have permitted N uptake to keep up with growth, resulting in higher concentrations than would have occurred if growth had been stimulated.

Even though total N concentrations were generally lower in blue grama than in the two C_3 species, buffer insoluble N concentrations were equal to or higher than

Table 2. Remobilization of total nonstructural carbohydrates and soluble N from root and crown tissues following defoliation. Treatment means were used to calculate the absolute amount and relative proportion of the available pools that were remobilized.

	Ambient CO_2		Elevated CO_2		Ambient CO_2		Elevated CO_2	
	Low N	High N	Low N	High N	Low N	High N	Low N	High N
Alfalfa	Total nonstructural carbohydrates				Soluble N			
Amount remobilized (mg/plant)	95	71	174	303	6.2	10.9	4.3	5.6
Relative remobilization (%)	50	53	70	76	48	65	45	43
Blue grama								
Amount remobilized (mg/plant)	16	19	9	10	0.7	4.0	0.3	1.9
Relative remobilization (%)	59	62	40	29	25	57	25	38
Western Wheatgrass								
Amount remobilized (mg/plant)	5	5	14	12	0.9	1.6	0.4	1.0
Relative remobilization (%)	66	60	70	54	51	64	26	33

the other species. The lower N concentrations in blue grama resulted from reductions in the two soluble fractions. The low molecular weight N fraction contains compounds that are readily available for remobilization to growing shoots, and indeed may be part of a N pool that is constantly cycling between roots and shoots (Simpson et al., 1982; Cooper and Clarkson, 1989) while the buffer soluble protein fraction contains storage proteins which can be quickly remobilized (Barber et al., 1996). Thus, with the exception of the high N-elevated CO_2 treatment, blue grama had a lower concentration of N in its roots and crowns at the time of defoliation that could potentially be remobilized than did the other two species (Table 3).

Root and crown soluble protein and low molecular weight N pools were depleted following defoliation while no significant decrease in insoluble N occurred (data not shown). Therefore, the soluble protein and low molecular weight N pools were combined to represent the soluble N pool that was readily available for remobilization (Fig. 2). The soluble N content of roots and crowns often increased immediately after defoliation even though the concentration remained the same or decreased. This suggests that changes in tissue concentration alone cannot be taken as an indication that remobilization has occurred.

On average, 41% of the soluble N was remobilized during the first 7 to 10 d following defoliation. When averaged across treatments, alfalfa had the greatest N remobilization (50%) while blue grama and western wheatgrass were similar with 36 and 38% remobilization, respectively (Table 2). The greatest amount of N remobilization occurred in the high N-ambient CO_2 treatment, where 57 to 65% of soluble N was remobilized in the three species. Low N and elevated CO_2 reduced both the amount and the percentage of soluble N that was remobilized.

The contribution of remobilized N to shoot growth was determined by comparing shoot N accumulation between two consecutive harvests with the amount of N lost from roots and crowns during the same period (Table 4). This assumes that all N lost from roots and crowns was translocated to shoots. A portion of the remobilized N, however, could have been lost from the plant through root exudation, which can increase following defoliation (Ofosu-Budu et al., 1995). Our estimates, therefore, suggest what the maximum contribution to regrowth could have been.

Reserve remobilization contributed more to alfalfa shoot growth than it did to either of the grasses (Table 4). Reserve N remobilization was least important in blue grama, which was consistent with the low levels of available reserves in this species. When averaged across species, N deficiency and elevated CO_2 reduced the contribution of remobilized N to shoot regrowth. The only exception was for blue grama, where N remobilization contributed more to regrowth under elevated rather than ambient CO_2 conditions in the low N treatment. In that case, the greater contribution of remobilized N to shoot growth resulted from decreased shoot N accumulation rather than from increased N remobilization. The decrease in N remobilization with low soil N was similar to previous reports, but the reduced contribution of remobilized N to regrowth was contrary to those same reports (Millard et al., 1990; Ourry et al., 1990; Thornton et al., 1994). We are not aware of any

Table 3. Concentration of root and crown nitrogen fractions at the time of defoliation. Concentration are expressed on a structural dry weight (sdw) basis. Data within a row followed by the same letter were not significantly different at $\alpha = 0.05$.

|	Ambient CO_2		Elevated CO_2		
	Low N	High N	Low N	High N	$LSD_{0.05}$
	mg N/g sdw				
Alfalfa					
Insoluble N	9.3a	9.2a	9.5a	9.4a	NS
Soluble proteins	5.8ab	6.4a	5.0b	5.2b	1.2
LMW† N	8.7b	12.3a	8.9b	8.9b	2.0
Total N	23.8b	27.9a	23.4b	23.5b	4.0
Blue grama					
Insoluble N	9.1b	12.8a	7.0c	13.9a	1.8
Soluble proteins	2.5b	3.6a	1.5c	3.6a	0.6
LMW N	2.9c	5.3b	3.0c	6.3a	0.8
Total N	14.5b	21.6a	11.5c	23.8a	2.5
Western wheatgrass					
Insoluble N	10.5a	10.1a	6.6b	11.4a	1.9
Soluble proteins	4.7b	6.3a	2.6c	2.7c	0.8
LMW N	4.9b	8.7a	4.6b	5.8b	1.5
Total N	20.2b	23.8a	13.8c	19.9b	2.9

† LMW = low molecular weight.
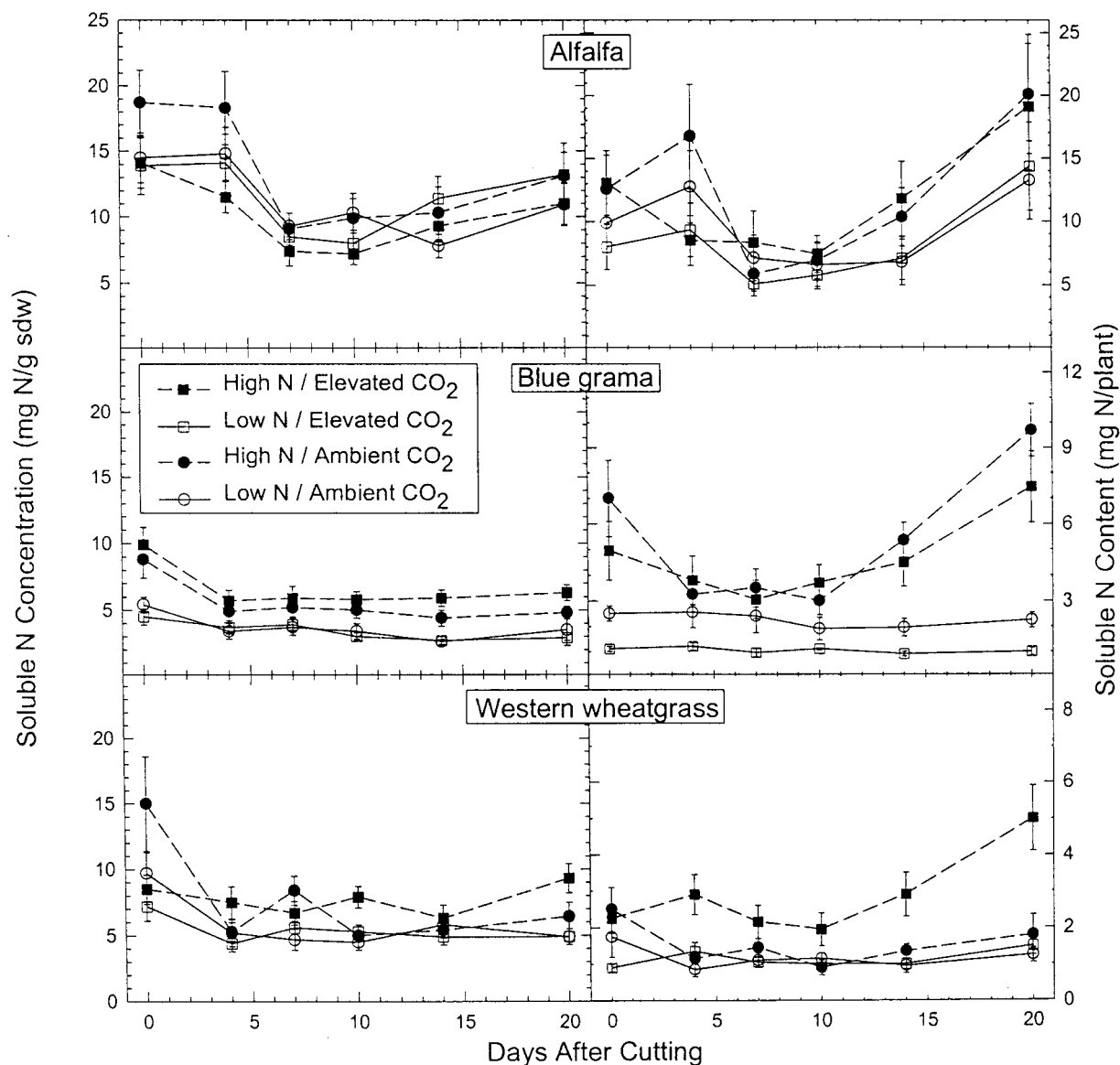
Fig. 2. Effect of N fertilization and elevated CO_2 on the concentration and content of soluble N compounds (buffer soluble protein and low molecular weight N) from roots and crowns of defoliated forages. Concentrations are expressed on a structural dry weight (sdw) basis. Error bars represent ±1 SE.

previous reports on how elevated CO_2 affects the relationship between N remobilization and regrowth.

Two factors could have contributed to the reduced contribution of remobilized N to shoot growth at low N and elevated CO_2. First, N accumulation rates were not suppressed as quickly by defoliation in the low N and elevated CO_2 treatments. This resulted in N accumulation rates that were often higher in low N and elevated CO_2 treatments for the first few days after defoliation (data not shown). Therefore, there was less need for remobilized N to meet regrowth demands. The low N results were consistent with previous reports where root growth and N uptake are less inhibited by defoliation when soil N supply is limited (Chapin and Slack, 1979; Richards, 1993). Second, the total amount and relative proportion of soluble N that was remobilized was generally less at low N and elevated CO_2 (Table 2), making less remobilized N available for regrowth.

Even though blue grama was the least reliant on reserve N remobilization for meeting shoot N requirements, blue grama shoot growth was more closely correlated with root and crown N supply and remobilization than were either of the C_3 species. Blue grama regrowth was correlated with root and crown soluble N content at the time of defoliation ($P = 0.02$) and with the proportion of soluble N that was remobilized ($P = 0.06$). Alfalfa regrowth was also correlated with soluble N content at the time of defoliation ($P = 0.06$). Thus, remobilization of N reserves was more closely correlated with shoot regrowth than was TNC remobilization.

By 20 d after cutting, root and crown TNC concentrations had generally recovered to levels that were similar to or greater than they were at the time of defoliation (Fig. 1). However, root and crown available N concentrations were still reduced after 20 d (Fig. 2). The longer period needed to replenish N reserves suggests that N

Table 4. Contribution of remobilized N from roots and crowns to shoot N pools. The percentages were derived by dividing the amount of soluble N (soluble protein and low molecular weight N) lost from roots and crowns between two consecutive harvests by the amount of N which accumulated in shoots over the same time period. Roots and crowns accumulated rather than lost N when the percentage is zero.

Species	Harvests	Ambient CO_2 Low N	Ambient CO_2 High N	Elevated CO_2 Low N	Elevated CO_2 High N
Alfalfa	0–4	0	0	0	76
	4–7	84	82	43	1
	7–10	7	0	0	4
Blue grama	0–4	0	47	0	15
	4–7	3	0	22	9
	7–10	5	5	0	0
Western Wheatgrass	0–4	67	75	0	0
	4–7	0	0	21	25
	7–10	0	24	3	0

rather than TNC availability would be most likely to determine when a pasture was ready to be regrazed. In the two C_3 species, N reserve concentrations approached the levels which existed at the time of defoliation more rapidly under elevated than under ambient CO_2. This was not true for blue grama. Western wheatgrass is more susceptible to grazing than is blue grama (Buwai and Trlica, 1977; Hart et al., 1993) and tends to decrease markedly in heavily grazed pastures. The improved ability of western wheatgrass to replenish root N reserves under elevated CO_2 could improve its ability to compete with blue grama under defoliation. This is supported by observations that elevated CO_2 also had a greater positive impact on western wheatgrass biomass production (Morgan, Skinner, and Hanson, 1998, unpublished data) and water use efficiency (Morgan et al., 1998). Future increases in atmospheric CO_2 could have a profound impact on species composition in the shortgrass steppe.

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Regrowth of White Clover after Chilling: Assimilate Partitioning and Vegetative Storage Proteins

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ABSTRACT

Under temperate climates, grassland species are subjected to overwintering which may significantly influence their early spring growth capacity. In white clover (*Trifolium repens* L.), it is known that overwintering capacity can differ among cultivars. Ability of this forage legume to recover from winter damage will, therefore, have a great influence on its persistence in grass–clover associations. Experiments were undertaken with two different white clover cultivars (Huia and AberHerald). Leaf appearance rate, dry matter distribution, ^{14}C assimilate partitioning, and vegetative storage protein accumulation were determined in plants subjected to a 4-wk chilling period (5/0°C, day/night) and subsequent warmer temperatures (15/10°C), and compared with control plants (20/15°C). Chilling treatment decreased leaf appearance rate, with AberHerald producing more leaves than Huia. This can be considered as a major aspect of cold adaptation strategy because leaf appearance rate controlled carbon acquisition. Low temperature increased dry matter partitioning to below-ground tissues. AberHerald allocated more assimilates to stolons than Huia. Accumulation of a 17.3-kDa protein, believed to act as a vegetative storage protein, also increased after the chilling treatment. Regrowth was characterized by rapid mobilization of the 17.3-kDa protein in stolons and by preferential carbon allocation to stolon apices. AberHerald showed a higher regrowth potential than Huia in view of its morphological and physiological characters which include carbon acquisition and assimilate partitioning patterns, favoring shoot regrowth and acquisition of stolon reserves.

I N TEMPERATE CLIMATES, the effects of overwintering on perennial cultivated plants deserve attention since the climatic events occurring during the winter greatly influence spring regrowth. This is particularly true in the case of white clover grown in association with grass because clover is susceptible to winter dam-

age (Harris et al., 1983; Frame and Newbould, 1986; Woledge et al., 1990). The principal consequences of low temperatures are losses of dry matter and stolon and bud death which can reduce spring regrowth potential. Competition between species is, therefore, exacerbated in early spring. Indeed, legume usually has a poor competitive ability with the associated grass. As a consequence, the persistence of the sward is reduced. Therefore, clovers which are tolerant to low winter temperatures and have good regrowth potential in spring are needed to improve the persistence of the pasture and reliability of yield from year to year.

Breeding programs have developed cold-tolerant cultivars from ecotypes collected in cool areas. Collins et al. (1991) have shown a considerable degree of genetic variation in the ability of white clover to overwinter and regrow in spring. AberHerald white clover, bred at IGER Aberystwyth from lines collected in the Zurich Oberland of the Swiss Alps, is known to be tolerant to low temperature and shows good spring regrowth potential (Rhodes and Fothergill, 1992; Collins et al. 1996; Caradus and Woodfield, 1997). However, we still do not clearly understand the plant characters and physiological mechanisms that explain the differential responses of cultivars to cold hardiness.

It is important to determine the morpho-physiological characters involved to identify more winter-hardy genotypes able to produce more biomass in early spring. We decided to investigate parameters related to carbon acquisition and partitioning (stolon morphogenesis, biomass, and partitioning of recently fixed carbon) and the fate of N reserves, as these factors are involved in regrowth after defoliation (Corre et al., 1996). We assumed that these processes are also involved in regrowth after previous exposure to cold temperatures. The involvement of starch and soluble sugars has already been investigated in overwintering and spring growth (Vez, 1961; Lüscher and Nösberger, 1992;

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Abbreviations: RSA, relative specific activity; SSW, specific stolon weight; VSP, vegetative storage proteins.